The Role of Iron Hydroxide as The Extracellular Matrix around Iron Bacteria

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The Role of Iron Hydroxide as The Extracellular Matrix around Iron Bacteria

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Abstract - Two types of biofloc containing mainly *Gallionella* spp. and *Leptothrix* spp. respectively have different Si/Fe intensity ratios characteristic of them. Soluble silicate addition makes the density of biofloc and that of microbes low. This study suggests that the binding force of biofloc influences the propagation of certain iron bacterial species.

I. Introduction

Microbial mat provides an inhabitable place for several types of microbe to whose surface such precipitations as silicate, alminosilicate, sulfur, calcium carbonate, iron hydroxide and manganese oxide adhere [1]. The precipitations form biologically and inorganically in microbial mat [2, 3]. How do these precipitations affect the propagatoion of the microbial species ? Preceding studies have not focused on the function of the precipitations in microbial mat. Therefore this study clarified the role of the precipitation, especially iron hydroxide in the microbial mat inhabited by iron bacteria.

II. Materials and methods

A. Undergroundwaters sampled from 2 drainages

The different types of undergroundwater were sampled from two drainages near parking area and circle building in surcharge storage pool located in Kanazawa University, Kakuma, Kanazawa on December 25th in 2002. Soon after sampling, the waters were sterilized by filtration using membrane filters of 0.2 μm pore size. The sterilized and no-sterilized waters were left not stirred in laboratory during 2 days. The water quality change of the no-sterilized water was measured by using pH meter (HORIBA D-21) and Electric Conductivity (EC) meter (HORIBA ES-14).

The bioflocs precipitated out of the no-sterilized water and the inorganic precipitation formed in the sterilized water were observed by the use of optical microscope (Nikon OPTIPHOT-2/LABOPHOT-2, EFD-3). DAPI (4', 6-diamidino-2-phenylindole) stained microbes in the biofloc were identified by bluish white light under UV light.

The chemical composition of sample surface was analysed by Scanning Electron Microscope (SEM: JEOL JSM-5200LV) equipped with Energy Dispersive X-ray spectroscopy (EDX: Philips EDAX PV 9800 STD). The analysis was operated at an accelerating voltage of 15 kV. A coating of cabon was evaporated over the surfaces of freeze-dried bioflocs mounted on stubs.

B. Addition of soluble silicate to undergroundwater

Such concentrations of sodium orthosilicate (KISHIDA Chemical) as 0, 0.5, 1.0, 1.5, 2.0 ppm were prepared by dissolving the reagent into sterilized undergroundwater collected from parking area on January 26th in 2003 and the no-sterilized water respectively. These water samples were left not stirred in laboratory for 2 days. The bioflocs precipitated out of the no-sterilized water and the inorganic precipitation formed in the sterilized water were observed by the use of optical microscope.

III. Results

A. Comparison between undergroundwaters from 2 drainages

Fig. 1 shows pH and EC changes of no-sterilized undergroundwaters sampled from 2 drainages near parking area and circle building. Immediately after sampling, the pH of two drainages indicates same value, but that of circle building increases more largely than that of parking area. The EC values of two drainages differ by about 100 μS/cm soon after sampling. The EC of parking area decreases more drastically than that of circle building.

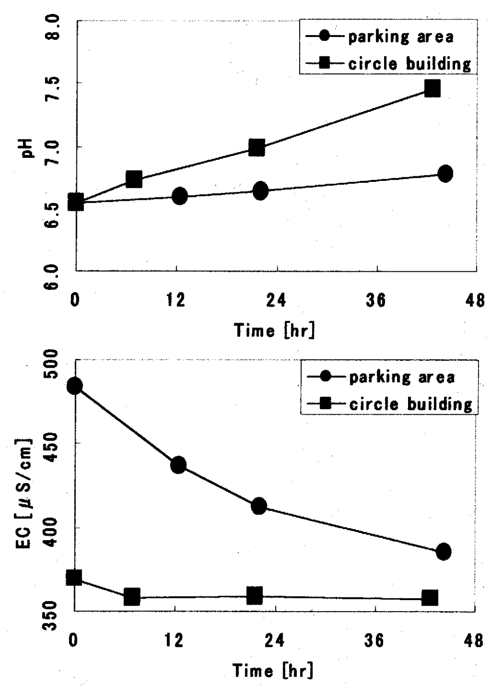

Fig. 1. The pH and EC changes of no-sterilized undergroundwater sampled from two drainages near parking area and circle building in surcharge storage pool located in Kanazawa University, Kakuma, Kanazawa.

Optical micrographs of the bioflocs precipitated out of no-sterilized undergroundwater sampled from parking area and the inorganic precipitation formed in the sterilized water are illustrated by Fig. 2. While the high density part of biofloc (Fig. 2: A-2) contains the aggregations of DAPI stained bacillus and coccus type bacteria (Fig. 2: A-1), the low density part (Fig. 2: B-2) have filamentous bacteria and a little number of bacillus and coccus type bacteria (Fig. 2: B-1). In the biofloc, *Gallionella* spp. (Fig. 2: C-1) and *Toxothrix* spp. (Fig. 2: C-2) are observed mainly. The inorganic precipitation consists of reddish brown grains of below 1 μm diameter (Fig. 2: D-1, D-2).

Optical micrographs of the bioflocs precipitated out of the no-sterilized water sampled from circle building and the inorganic precipitation formed in the sterilized water are illustrated by Fig. 3. While the high density part of biofloc (Fig. 3: A-2, B-2) contains bacillus and coccus type bacteria (Fig. 3: A-1, B-1), the low density part (Fig. 3: A-2, B-2) has filamentous bacteria (Fig. 3: A-1, B-1). In the biofloc, *Leptothrix* spp. (Fig. 3: C-1) and *Toxothrix* spp. (Fig. 3: C-2) are observed mainly. The inorganic precipitation consists of reddish brown flakes of above 20 x 20 μm size (Fig. 3: D-1, D-2).

Several dots in Fig. 4 show the ratios of Si integral intensity to Fe integral intensity calculated from the chemical compositions of sample surfaces analyzed by SEM-EDX. The Si/Fe ratios of the sample surface numbers 1, 2, 3 range over a similar region just as do those of 4, ,5, 6. The biofloc and the inorganic precipitation of parking area have less Si content than those of circle building.

B. *Variation of biofloc and inorganic precipitation with silicate addition*

Fig. 5 illustrates the variation of the biofloc precipitated out of no-sterilized undergroundwater sampled from parking area and the inorganic precipitation formed in sterilized water with soluble silicate concentration. According to increasing of the silicate concentration, the density of biofloc decreases (Fig. 5: A-1, B-1, C-1, D-1, E-1) , that of bacillus and coccus type bacteria decreases (Fig. 5: A-2, B-2, C-2, D-2, E-2) and the size of brown flakes increases (Fig. 5: A-3, B-3, C-3, D-3, E-3).

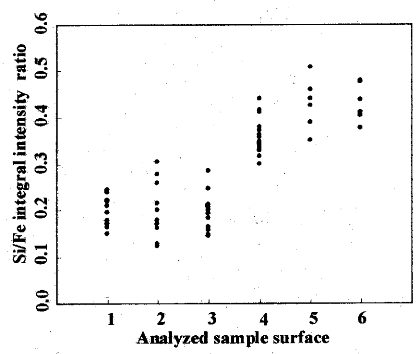

Fig. 4. The ratios of Si integral intensity to Fe integral intensity calculated from the chemical compositions of sample surfaces analyzed by SEM-EDX. The number labels on an abscissa axis indicate such analyzed sample surfaces as the inorganic precipitation formed in sterilized undergroundwater of parking area (1), the ribbon-like secretions of *Gallionella* spp. in biofloc precipitated from no-sterilized water of parking area (2), the substances other than *Gallionella* spp. in biofloc precipitated from no-sterilized water of parking area (3), the inorganic precipitation formed in sterilized water of circle building (4), the sheaths of *Leptothrix* spp. in biofloc precipitated from no-sterilized water of circle building (5) and the substances other than *Leptothrix* spp. in biofloc precipitated from no-sterilized water of circle building (6).

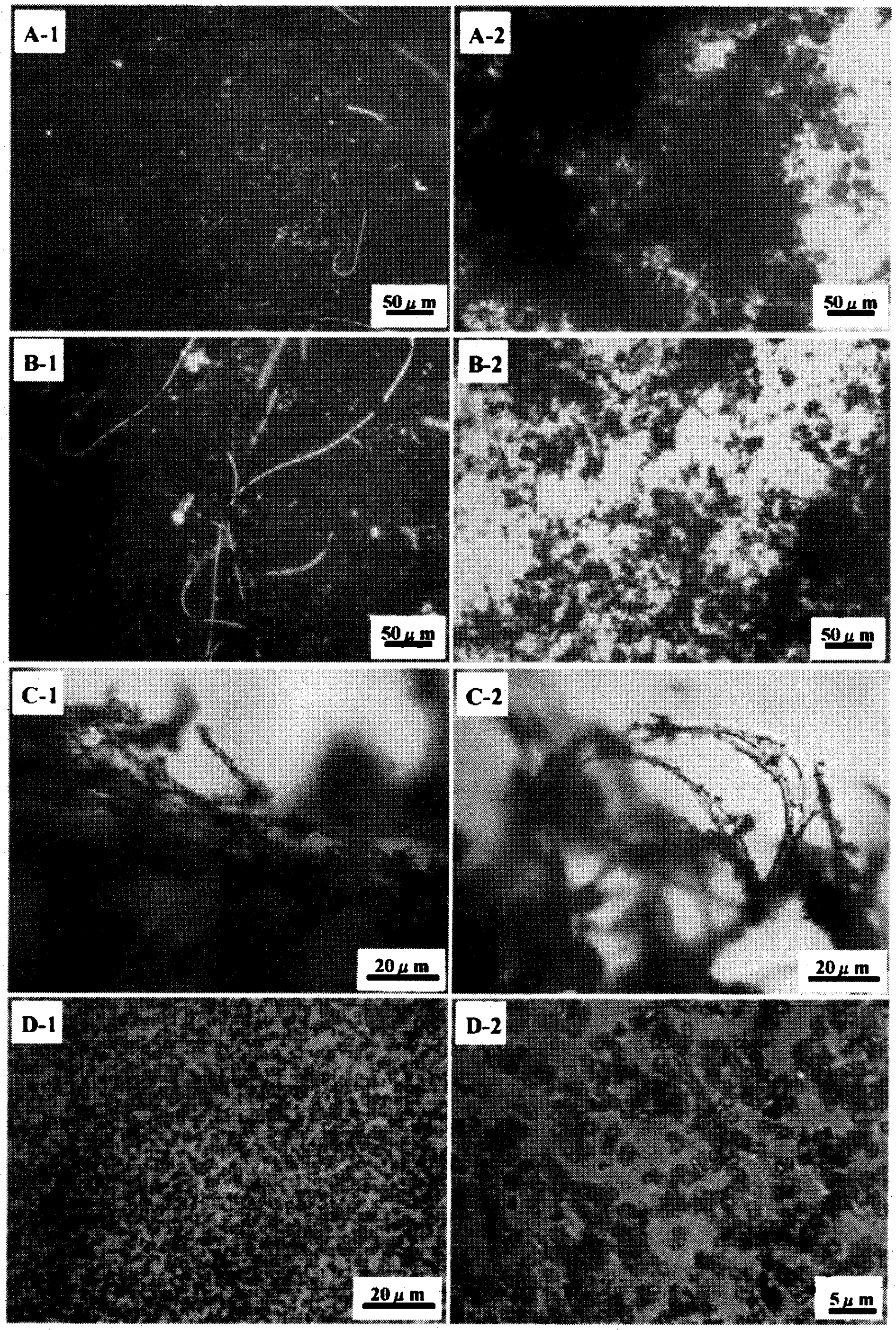

Fig. 2. Optical micrographs of the biofloc precipitated out of no-sterilized undergroundwater sampled from parking area (A-1, A-2, B-1, B-2, C-1, C-2) and the inorganic precipitation formed in the sterilized water (D-1, D-2). The two micrographs of A-1, B-1 are taken under UV light, whereas others are done under visible light.

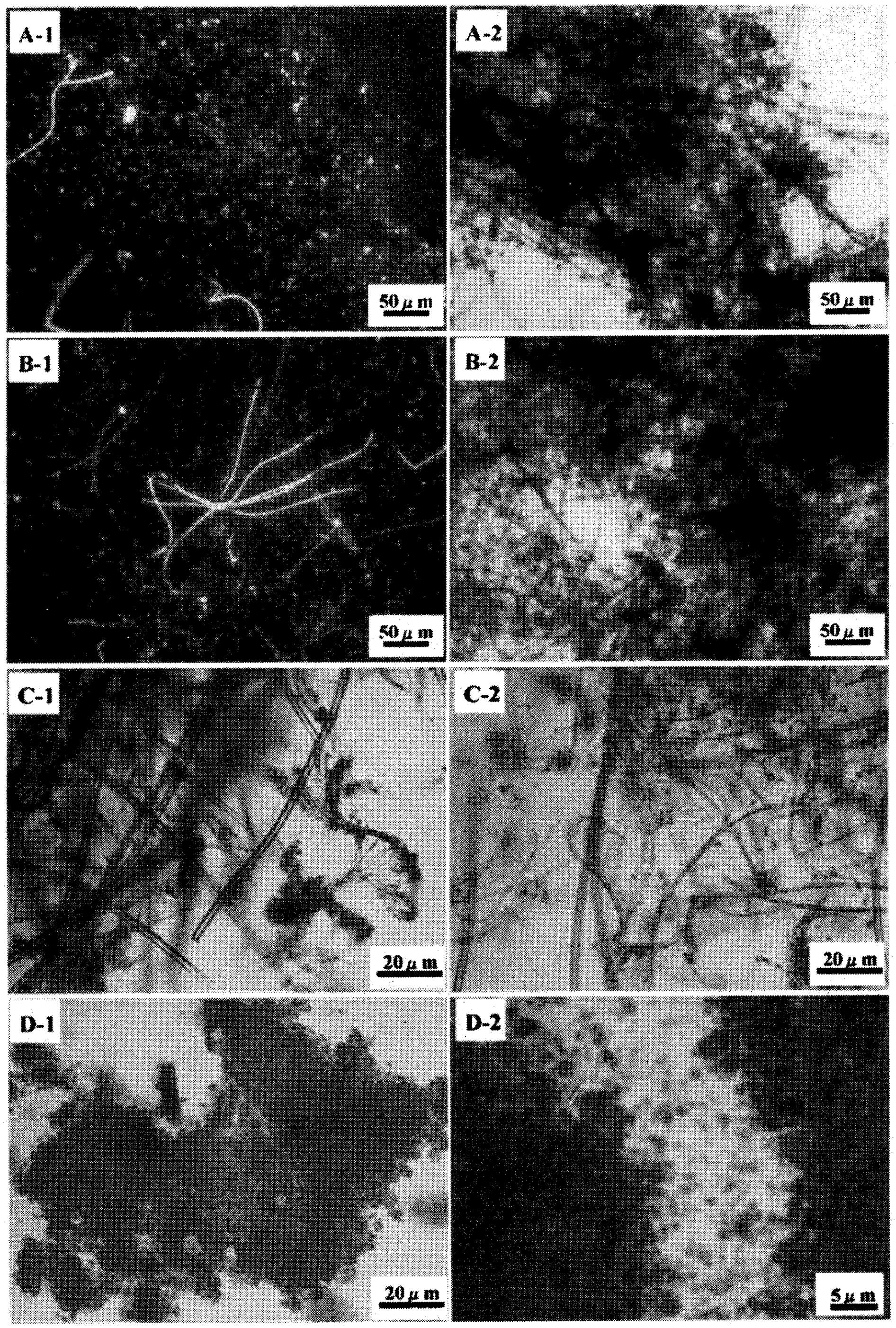

Fig. 3. Optical micrographs of the biofloc precipitated out of the no-sterilized undergroundwater sampled from circle building (A-1, A-2, B-1, B-2, C-1, C-2) and the inorganic precipitation formed in the sterilized water (D-1, D-2). The two micrographs of A-1, B-1 are taken under UV light, whereas the others are done under visible light.

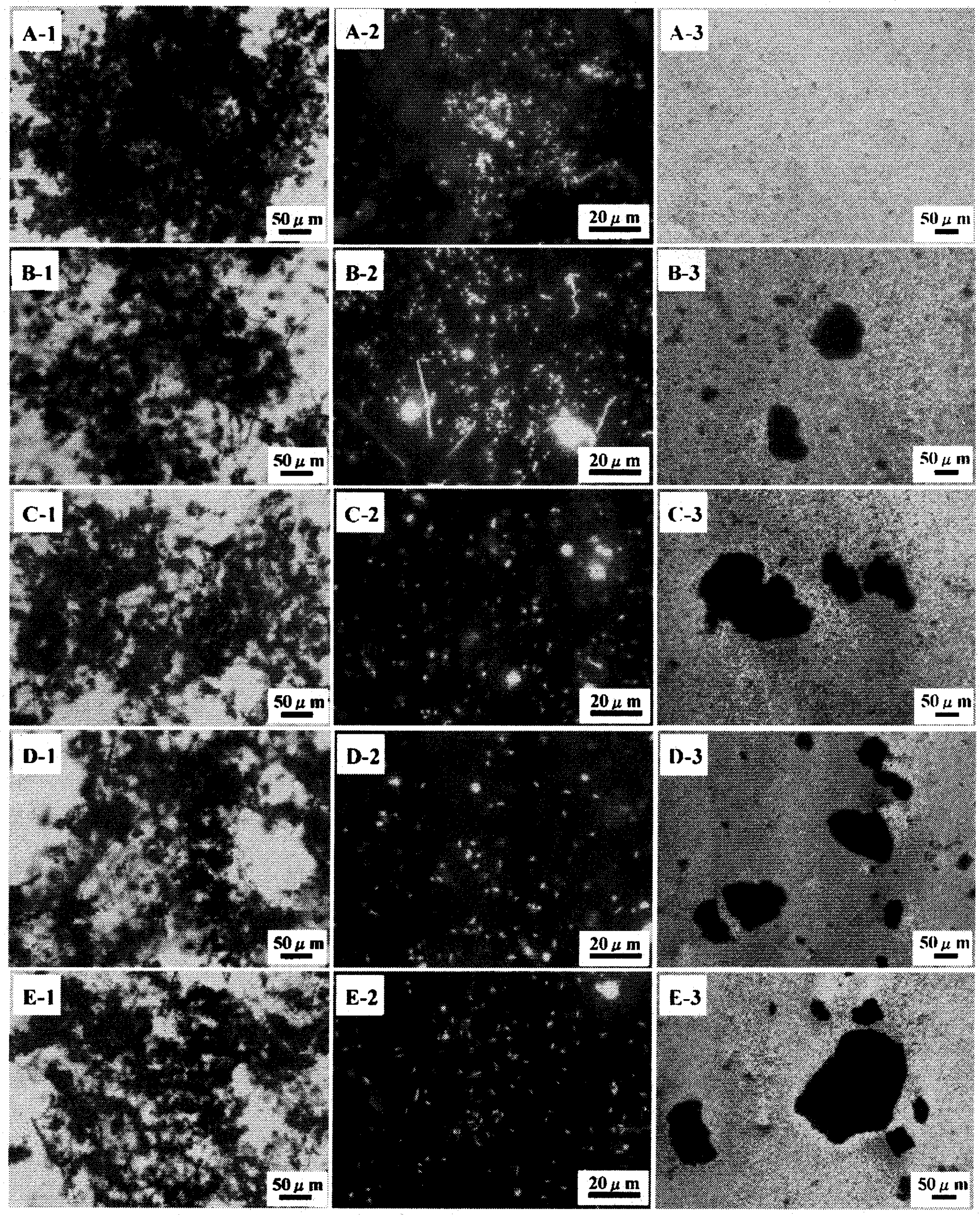

Fig. 5. Variation of the biofloc precipitated out of no-sterilized undergroundwater sampled from parking area (A-1, A-2, B-1, B-2, C-1, C-2, D-1, D-2, E-1, E-2) and the inorganic precipitation formed in sterilized water sampled from parking area (A-3, B-3, C-3, D-3, E-3) with soluble silicate concentrations. Such concentrations of sodium orthosilicate as 0, 0.5, 1.0, 1.5, 2.0 ppm were prepared by dissolving the reagent into the water (A 0.0 ppm, B 0.5 ppm, C 1.0 ppm, D 1.5 ppm, E 2.0 ppm). The five micrographs of A-2, B-2, C-2, D-2, E-2 are taken under UV light, whereas the others are done under visible light.

IV. Discussion

The Fe and Si peaks in the chemical composition of biofloc are mainly attributed to iron hydroxide and silicate respectively. Generally the surface of colloidal iron hydroxide is positively charged and silicate ion is negatively charged. The inorganic precipitation formed in sterilized undergroundwater of parking area has less Si content than that of circle building (Fig. 4). While the inorganic precipitation of parking area doesn't aggregate (Fig. 2: D-1, D-2), that of circle building contains large flakes (Fig. 3: D-1, D-2). Those suggest that the inorganic precipitation of parking area is more positively charged than that of circle building.

The negatively charged surface of microbe adsorbs the positively charged inorganic precipitation. The decreases of biofloc density (Fig. 5: A-1, B-1, C-1, D-1, E-1) and of bacillus and coccus type bacteria (Fig. 5: A-2, B-2, C-2, D-2, E-2) with silicate concentration imply that each microbes are connected with iron hydroxide and the silicate addtion releases the each microbes from binding force. The binding force may be one of key factors influencing the propagation of certain iron bacterial species.

References

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